January 2013

ipsogen® NPM1 Muta*Screen* Handbook

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For detection of NPM1 mutations and identification of mutation types A, B, and D

For research use only. Not for use in diagnostic procedures.

For use with Rotor-Gene® Q, Applied Biosystems® 7500 Real-Time PCR System, ABI PRISM® 7900HT SDS, and LightCycler® 480 instruments

REF 677013

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Intended Use

The *ipsogen* NPM1 Muta*Screen* Kit is intended for research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Principle of the Procedure

The *ipsogen* NPM1 Muta*Screen* Kit combines two techniques to screen for the presence of mutations in the target gene. The real-time quantitative PCR (qPCR) double-dye oligonucleotide hydrolysis principle uses specific primers and an internal double-dye probe with a reporter and a quencher (FAM™-TAMRA™) for the amplification reactions. In addition, a 3'-end modified phosphate oligonucleotide is used that perfectly matches the wild-type NPM1 gene and does not allow polymerization.

During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. The DNA polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, polymerization of the strand continues, and a fluorescent signal is released. The 3' end of the probe is blocked to prevent extension of the probe during PCR (see Figure 1).

The phosphate oligonucleotide enhances specificity of the PCR reaction by competition with one PCR primer for a common target site. When the PCR template contains the wild-type sequence, the phosphate oligonucleotide will dominate over PCR primer binding due to higher affinity. There is no extension by the DNA polymerase, and no amplification is observed. When the mutated sequence is present, PCR primer binding will dominate over phosphate oligonucleotide binding and amplification will proceed.

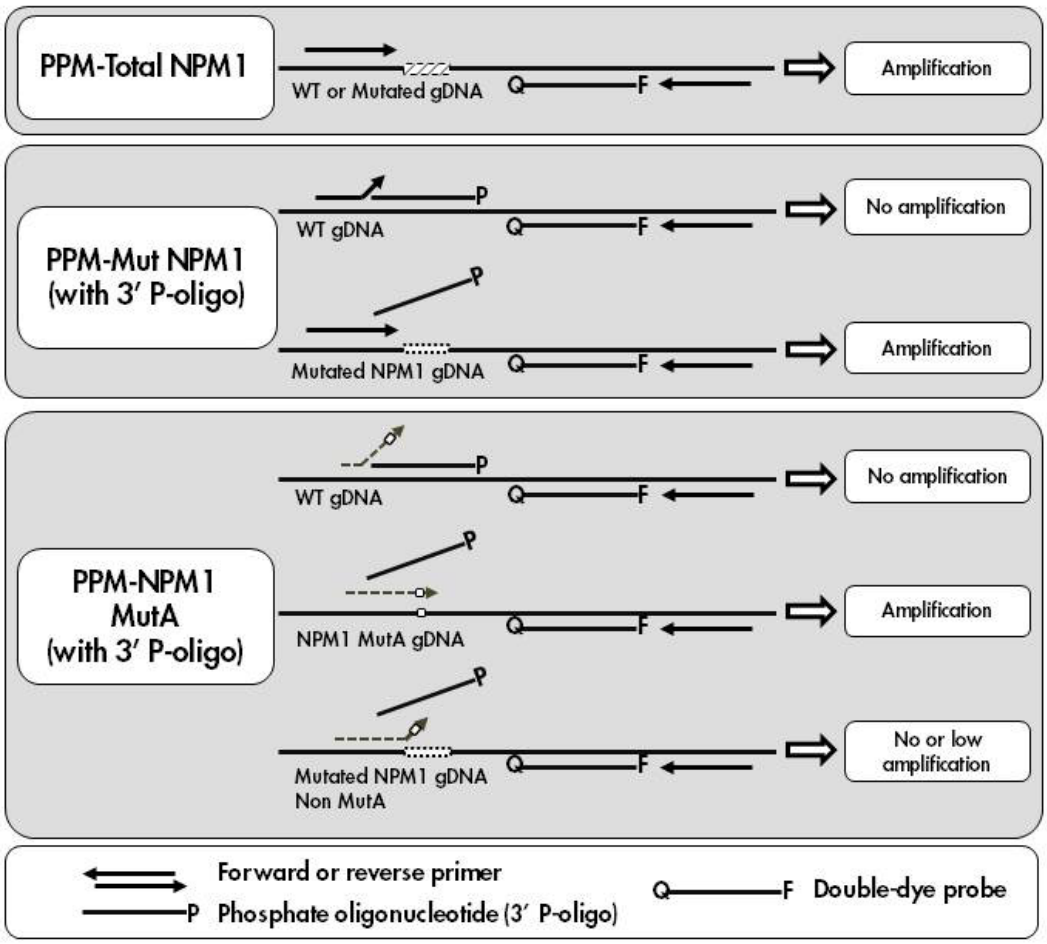

Figure 1. Results obtained with the primers and probe mixes in the *ipsogen* NPM1 *MutaScreen* Kit. The same principle shown to detect NPM1 MutA applies for NPM1 MutB and NPM1 MutD.

Materials Provided

Kit contents

***ipsogen* NPM1 *MutaScreen* Kit**		**(24)**
Catalog no.		**677013**
Number of reactions		**24**
NPM1 wild-type control (wild-type NPM1 genomic DNA)	WTC	300 µl
NPM1 mutated positive control (NPM1 MutA/B/D genomic DNA)	Mut-PC	300 µl
Primers and Probe Mix Total NPM1*	PPM-Total NPM1 25x	90 µl
Primers and Probe Mix Mutated NPM1†	PPM-Mut NPM1 25x	90 µl
Primers and Probe Mix NPM1 MutA‡	PPM-NPM1 MutA 25x	90 µl
Primers and Probe Mix NPM1 MutB§	PPM-NPM1 MutB 25x	90 µl
Primers and Probe Mix NPM1 MutD¶	PPM-NPM1 MutD 25x	90 µl

* Mix of specific reverse and forward primers for total NPM1 (wild-type and mutated) plus a specific FAM–TAMRA probe.

† Mix of specific reverse and forward primers (including phosphate primer) for all mutations of NPM1 plus a specific FAM–TAMRA probe.

‡ Mix of specific reverse and forward primers (including phosphate oligonucleotide) for the specific detection of NPM1 MutA plus a specific FAM–TAMRA probe.

§ Mix of specific reverse and forward primers (including phosphate oligonucleotide) for the specific detection of NPM1 MutB plus a specific FAM–TAMRA probe.

¶ Mix of specific reverse and forward primers (including phosphate oligonucleotide) for the specific detection of NPM1 MutD plus a specific FAM–TAMRA probe.

Note: Vortex and briefly centrifuge the controls and the primers and probe mixes before use.

Materials Required but Not Provided

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Reagents

- Nuclease-free PCR grade water
- Buffer and *Taq* DNA polymerase: The recommended reagents are TaqMan® Universal PCR Master Mix (Master Mix PCR 2x) (Life Technologies, cat. no. 4304437)

Consumables

- Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters
- 0.5 ml or 1.5 ml nuclease-free PCR tubes
- Ice

Equipment

- Microliter pipet*dedicated for PCR (1–10 µl; 10–100 µl; 100–1000 µl)
- Benchtop centrifuge* with rotor for 0.5 ml/1.5 ml reaction tubes and microplates (capable of attaining 13,000–14,000 rpm)
- Real-time PCR instrument:* Rotor-Gene Q 5plex HRM® or other Rotor-Gene instrument; LightCycler 480; Applied Biosystems 7500 Real-Time PCR System; ABI PRISM 7900HT SDS; and associated specific material
- Spectrophotometer*

Warnings and Precautions

When working with chemicals, always wear a suitable laboratory coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Discard sample and assay waste according to your local safety regulations.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

General precautions

Use of qPCR tests require good laboratory practices, including maintenance of equipment, that are dedicated to molecular biology and is compliant with applicable regulations and relevant standards.

This kit is intended for research use. Reagents and instructions supplied in this kit have been tested for optimal performance. Further dilution of the reagents or alteration of incubation times and temperatures may result in erroneous or discordant data. Primers and probe mix (PPM) reagents may be altered if exposed to light. All reagents are formulated specifically for use with this kit. For optimal performance of the kit, no substitutions should be made.

Use extreme caution to prevent:

- DNase contamination that might cause degradation of the template DNA
- DNA or PCR carryover contamination resulting in false positive signal

We therefore recommend the following.

- Use nuclease-free labware (e.g., pipets, pipet tips, reaction vials) and wear gloves when performing the assay.
- Use fresh aerosol-resistant pipet tips for all pipetting steps to avoid cross-contamination of the samples and reagents.
- Prepare pre-PCR master mix with dedicated material (pipets, tips, etc.) in a dedicated area where no DNA matrices (DNA, plasmid, or PCR products) are introduced. Add template in a separate zone (preferably in a separate room) with specific material (pipets, tips, etc.).

Reagent Storage and Handling

The kits are shipped on dry ice and must be stored at –30°C to –15°C upon receipt.

- Minimize exposure to light of the primers and probe mixes (PPM tubes).
- Gently mix and centrifuge the tubes before opening.
- Store all kit components in original containers.

These storage conditions apply to both opened and unopened components. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

Expiration dates for each reagent are indicated on the individual component labels. Under correct storage conditions, the product will maintain performance until the expiration date printed on the label.

There are no obvious signs to indicate instability of this product. However, positive and negative controls should be run simultaneously with unknown specimens.

Procedure

Sample DNA preparation

Genomic DNA should be obtained either from whole blood, purified peripheral blood lymphocytes of whole blood, polynuclear cells, or granulocytes. For comparable results it is recommended that the same cellular fraction and DNA extraction method are used. DNA extraction can be performed using a commercially available kit or a home-brew method.

DNA quantity should be determined by measuring the optical density (OD) of the sample at 260 nm and DNA quality can be determined either by spectrophotometry or gel* electrophoresis.

- The OD_{260}/OD_{280} ratio should be 1.7–1.9 and smaller ratios than this may indicate protein contamination or the presence of organic chemicals.
- Electrophoretic analysis on a 0.8–1.0% agarose gel* should allow the visualization of the isolated DNA as a distinct band of approximately 20 kb (a slight smear will give acceptable results).

The resultant DNA will need to be diluted to a concentration of 5 ng/µl in 1x TE buffer* at pH 8.0 and then stored at +4 to +8°C for 1 week or at –20°C if longer term storage is required.

The qPCR reaction is optimized for DNA samples containing 25 ng purified genomic DNA. An amount of 250 ng of genomic DNA is needed per sample.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Protocol: qPCR on Rotor-Gene Q 5plex HRM instruments with 72-tube rotor

Using this instrument, we recommend performing all measurements in duplicate, as indicated in Table 1.

Table 1. Number of reactions for Rotor-Gene Q instruments with 72-tube rotor

Samples	Reactions
With the total NPM1 primers and probe mix (PPM-Total NPM1)	
With the mutated NPM1 primers and probe mix (PPM-Mut NPM1)	
With the NPM1 MutA primers and probe mix (PPM-NPM1 MutA)	
With the NPM1 MutB primers and probe mix (PPM-NPM1 MutB)	
With the NPM1 MutD primers and probe mix (PPM-NPM1 MutD)	
n DNA samples	n x 2 reactions
2 DNA controls	4 reactions: wild-type control (WTC) and mutated positive control (Mut-PC), each one tested in duplicate
Water control	2 reactions

Sample processing on Rotor-Gene Q instruments with 72-tube rotor

We recommend testing 4 DNA samples in the same experiment, 14 reactions with each primers and probe mix, to optimize the use of controls and the primers and probe mixes.

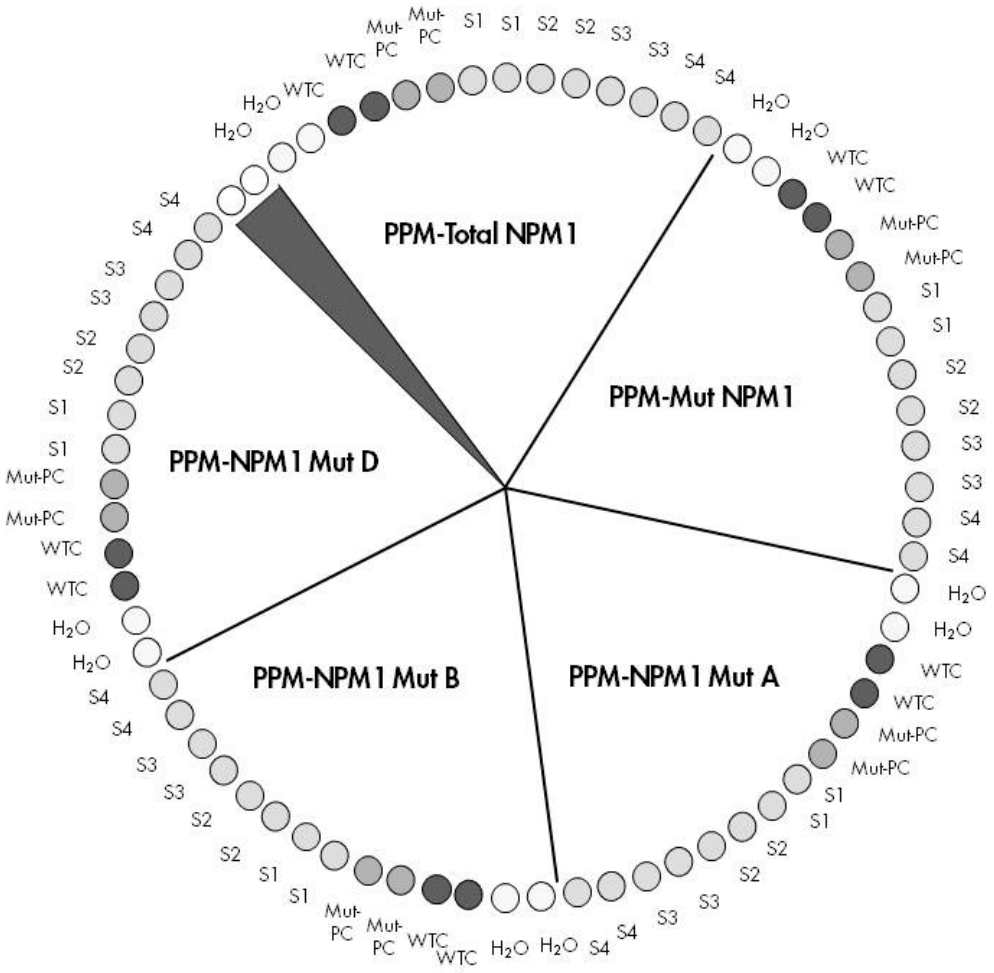

Figure 3. Suggested rotor setup for an experiment with the *ipsogen* NPM1 MutaScreen Kit. WTC: wild-type NPM1positive control; **Mut-PC**: mutated NPM1 positive control; **S**: DNA sample; **H_2O**: water control.

Note: Take care to always place a sample to be tested in position 1 of the rotor. Otherwise, during the calibration step, the instrument will not perform calibration, and incorrect fluorescence data will be acquired.

Fill all other positions with empty tubes.

qPCR on Rotor-Gene Q instruments with 72-tube rotor

Note: Perform all steps on ice.

Procedure

1. **Thaw all necessary components and place them on ice.**
2. **Prepare the following qPCR mix for each primers and probe mix according to the number of samples being processed.**

   All concentrations are for the final volume of the reaction.

Table 2 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix (PPM-Total NPM1, PPM-Mut NPM1, PPM-NPM1 MutA, PPM-NPM1 MutB, or PPM-NPM1 MutD). Extra volumes are included to compensate for pipetting error.

Table 2. Preparation of qPCR mix for PPM-Total NPM1, PPM-Mut NPM1, PPM-NPM1 MutA, PPM-NPM1 MutB, or PPM-NPM1 MutD

Component	1 reaction (µl)	Pre-mix 14 + 1 reactions (µl)	Final concentration
TaqMan Universal PCR Master Mix, 2x	12.5	187.5	1x
Primers and probe mix, PPM 25x	1.0	15	1x
Nuclease-free PCR grade water	6.5	97.5	–
Sample (to be added at step 4)	5.0	5 each	–
Total volume	25.0	25 each	–

3. **Dispense 20 µl of the qPCR pre-mix per tube.**
4. **Add 5 µl of the material to be quantified (25 ng sample genomic DNA or control) in the corresponding tube (total volume 25 µl).**
5. **Mix gently, by pipetting up and down.**
6. **Place the tubes in the thermal cycler according to the manufacturer recommendations.**
7. **Program the Rotor-Gene Q instrument with the thermal cycling program as indicated in Table 3.**

Table 3. Temperature profile

Mode of analysis	Quantitation
Hold	Temperature: 50 deg Time: 2 mins
Hold 2	Temperature: 95 deg Time: 10 mins
Cycling	40 times 95 deg for 15 secs 60 deg for 1 min 30 secs with acquisition of FAM fluorescence in channel Green: Single

8. **For Rotor-Gene Q instruments, select "Slope Correct" for the analysis. We recommend setting the threshold at 0.03. Start the thermal cycling program, as indicated in Table 3.**

Protocol: qPCR on Applied Biosystems 7500 and ABI PRISM 7900HT instruments, and LightCycler 480 instrument

Using 96-well-plate qPCR equipment, we recommend performing all measurements in duplicate, as indicated in Table 4.

Table 4. Number of reactions using 96-well plate qPCR equipment

Samples	Reactions
With the total NPM1 primers and probe mix (PPM-Total NPM1)	
With the mutated NPM1 primers and probe mix (PPM-Mut NPM1)	
With the NPM1 MutA primers and probe mix (PPM-NPM1 MutA)	
With the NPM1 MutB primers and probe mix (PPM-NPM1 MutB)	
With the NPM1 MutD primers and probe mix (PPM-NPM1 MutD)	
n DNA samples	n x 2 reactions
2 DNA controls	4 reactions: wild-type control (WTC) and mutated positive control (Mut-PC), each one tested in duplicate
Water control	2 reactions

Sample processing on Applied Biosystems 7500 and ABI PRISM 7900HT instruments, and LightCycler 480 instrument

We recommend testing 4 DNA samples in the same experiment, 14 reactions with each primers and probe mix, to optimize the use of controls and the primers and probe mixes.

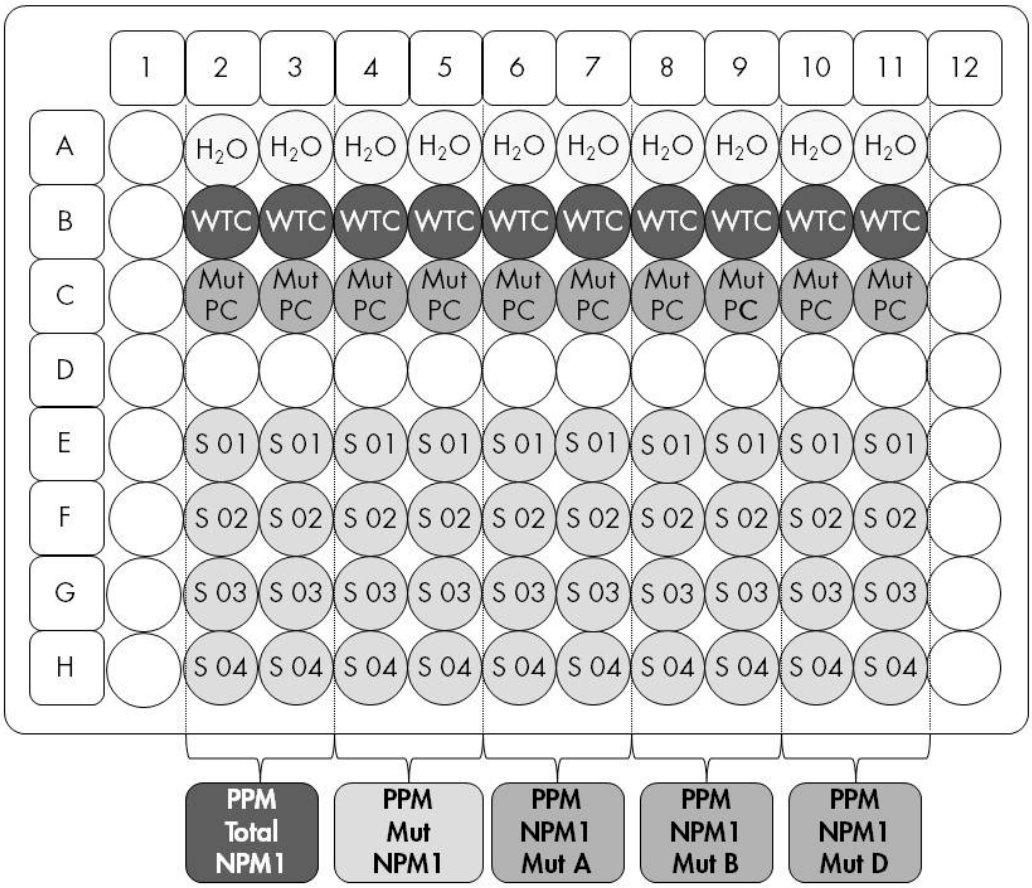

Figure 4. Suggested plate setup for an experiment with the *ipsogen* NPM1 Muta*Screen* Kit. WTC: wild-type NPM1positive control; **Mut-PC**: mutated NPM1 positive control; **S**: DNA sample; **H_2O**: water control. **S**: DNA sample; **H_2O**: water control

qPCR on Applied Biosystems 7500 and ABI PRISM 7900HT instruments, and LightCycler 480 instrument

Note: Perform all steps on ice.

Procedure

1. **Thaw all necessary components and place them on ice.**
2. **Prepare the following qPCR mix for each primers and probe mix according to the number of samples being processed.**

   All concentrations are for the final volume of the reaction.

   Table 5 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix (PPM-Total NPM1, PPM-Mut NPM1, PPM-NPM1 MutA, PPM-NPM1 MutB, or PPM-NPM1 MutD). Extra volumes are included to compensate for pipetting error.

Table 5. Preparation of qPCR mix for PPM-Total NPM1, PPM-Mut NPM1, PPM-NPM1 *MutA*, PPM-NPM1 *MutB*, or PPM-NPM1 *MutD*

Component	1 reaction (µl)	Pre-mix 14 + 1 reactions (µl)	Final concentration
TaqMan Universal PCR Master Mix, 2x	12.5	187.5	1x
Primers and probe mix, PPM 25x	1.0	15	1x
Nuclease-free PCR grade water	6.5	97.5	–
Sample (to be added at step 4)	5.0	5 each	–
Total volume	25.0	25 each	–

3. **Dispense 20 µl of the qPCR pre-mix per well.**
4. **Add 5 µl of the material to be quantified (25 ng sample genomic DNA or control) in the corresponding well (total volume 25 µl).**
5. **Mix gently, by pipetting up and down.**
6. **Close the plate and briefly centrifuge (300 x *g*, approximately 10 seconds).**
7. **Place the plate in the thermal cycler according to the manufacturer recommendations.**
8. **Program the thermal cycler with the thermal cycling program and set the instrument for the acquisition of dual labeled FAM fluorescent probe as indicated in Table 6 for Applied Biosystems 7500 and ABI PRISM 7900HT instruments, or Table 7 for the LightCycler 480 instrument.**

Table 6. Temperature profile for Applied Biosystems 7500, ABI PRISM 7000, ABI PRISM 7700, or ABI PRISM 7900HT instruments

Mode of analysis	Standard Curve — Absolute Quantitation
Hold	Temperature: 50°C Time: 2 minutes
Hold 2	Temperature: 95°C Time: 10 minutes
Cycling	40 times 95°C for 15 seconds 60°C for 1 minute 30 seconds with acquisition of FAM fluorescence; quencher: TAMRA

Table 7. Temperature profile for LightCycler 480 instrument

Mode of analysis	Absolute Quantification ("Abs Quant")
Detection formats	Select "Simple Probe" in the Detection formats window
Hold	Temperature: 50°C Time: 2 minutes
Hold 2	Temperature: 95°C Time: 10 minutes
Cycling	40 times 95°C for 15 seconds 60°C for 1 minute 30 seconds with acquisition of FAM fluorescence corresponding to (483–533 nm) for LC version 01 and (465–510 nm) for LC version 02

9. For the Applied Biosystems 7500 and ABI PRISM 7900HT instruments, follow step 9a. For the LightCycler 480 instrument, follow step 9b.

9a. Applied Biosystems 7500 and ABI PRISM 7900HT instruments: We recommend a threshold set at 0.1 and a baseline set between cycles 3 and 15. Start the cycling program, as indicated in Table 6.

9b. LightCycler 480: We recommend a Fit point analysis mode with background at 2.0 and threshold at 2.0. Start the thermal cycling program, as indicated in Table 7.

Results

Calculate the mean threshold cycle (C_T) value obtained with each primers and probe mix for the controls (NPM1 wild-type control and mutated NPM1 positive control) and for each sample. If one of the duplicates of a sample has an "undetermined" value, we recommend retesting the sample.

Quality control using C_T values of controls

The NPM1 wild-type control (WTC) and the mutated NPM1 positive control (Mut-PC) allow an experiment to be qualified.

- The NPM1 wild-type control must be detected with only PPM-Total NPM1.
- The mutated NPM1 positive control must be detected with all PPMs.

The entire experiment is rejected if both conditions are not met.

Water controls

Water controls (non-template controls) should give zero C_T values for all primers and probe mixes.

A positive water control results from a cross-contamination. See "Troubleshooting guide", below, to find a solution.

Sample input validation

A sample's input must be validated before interpretation. The validation interval is determined with these calculations.

C_T Total NPM1 (WTC): C_T value of the NPM1 wild-type control with PPM-Total NPM1

C_T Total NPM1 (Mut-PC): C_T value of the mutated NPM1 positive control with PPM-Total NPM1

C_T Total NPM1 (Sample): C_T value of a sample with PPM-Total NPM1

The value of **C_T Total NPM1 (Sample)** must be within the following interval:

[C_T Total NPM1 (WTC); C_T Total NPM1 (Mut-PC) + 2.3]

The sample is positive for an NPM1 mutation if a C_T value is obtained with the primers and probe mix PPM-Mut NPM1. This is **C_T Mut NPM1 (Sample)**.

The NPM1 mutation type of a sample, mutA, MutB, or MutD, is determined by the C_T values obtained with PPM-NPM1 MutA, PPM-NPM1 MutB, and PPM-NPM1 MutD. The mutation type is indicated by the result with the lowest C_T value, providing that C_T value respects the following rule:

$$\mathbf{C_T\ _{NPM1\ MutA,\ MutB,\ or\ MutD}\ (Sample) \leq C_T\ _{Mut\ NPM1}\ (Sample) + 4.4}$$

A summary of the rules of sample validation and analysis is given in Table 8.

Table 8. Successive rules applied for run validation and results analysis

No.	Stage		Rule
1	Quality control	Water controls	Must give zero C_T values
2		NPM1 wild-type control	Must be detected with only PPM-total NPM1
3		Mutated NPM1 positive control	Must be detected with all primer and probe mixes
4	Sample input	Input interval	[C_T Total NPM1 (WTC);C_T Total NPM1 (Mut-PC)+2.3]
5		Sample validation	The value of C_T Total NPM1 (Sample) must be within the input interval
6	Analysis	Mutation detection	A sample is positive for an NPM1 mutation if a C_T value is obtained with PPM-Mut NPM1: C_T Mut NPM1 (Sample)
7		Mutation type detection	The NPM1 mutation type of a sample is determined by the lowest C_T value obtained with PPM-NPM1 MutA, PPM-NPM1 MutB, and PPM-NPM1 MutD providing C_T NPM1 MutA, MutB, or MutD (Sample) ≤ C_T Mut NPM1 (Sample) + 4.4

Step by step interpretation of results

Interpretation is shown of results from 9 samples plus controls analyzed with the *ipsogen* NPM1 MutaScreen Kit.

Quality control

Quality control using C_T values of controls is shown in Table 9.

Table 9. Run validation on controls

	Mean C_T values with primers and probe mixes				
Control	**Total NPM1**	**Mut NPM1**	**NPM1 MutA**	**NPM1 MutB**	**NPM1 MutD**
Water (negative)	Undetected	Undetected	Undetected	Undetected	Undetected
NPM1 WTC	23.84	Undetected	Undetected	Undetected	Undetected
NPM1 Mut-PC	26.9	30.49	31.88	32.82	33.09

Quality control shows that the run is valid. Conditions of rules 1, 2, and 3 have been met:

- Rule 1: Water control is undetected with all primer and probe mixes.
- Rule 2: NPM1 WTC is detected with only PPM-Total NPM1.
- Rule 3: NPM1-Mut PC is detected with all primer and probe mixes.

Input interval

Determination of the input interval using C_T values of positive controls is shown.

Input interval **$[C_{T\ \text{Total NPM1}}$ (WTC); $C_{T\ \text{Total NPM1}}$ (Mut-PC) + 2.3]**

[23.84; 26.9 + 2.3]

Input interval [23.84; 29.2]

- Rule 4: The input interval for sample validation is determined.

Sample validation

Sample input validation using the input interval is shown in Table 10.

Table 10. Sample input validation

	Mean C_T values with primers and probe mixes				
Sample	**Total NPM1**	**Mut NPM1**	**NPM1 *MutA***	**NPM1 *MutB***	**NPM1 *MutD***
1	26.46	27.66	28.29	Undetected	38.37
2	26.12	32.73	32.78	Undetected	Undetected
3	27.68	29.12	Undetected	29.79	Undetected
4	28.78	34.92	Undetected	33.57	Undetected
5	26.36	27.49	36.28	Undetected	27.96
6	26.56	33.73	Undetected	Undetected	33.54
7	25.81	27.06	37.23	38.7	Undetected
8	26.89	27.87	38.91	Undetected	Undetected
9	25.64	Undetected	Undetected	Undetected	Undetected

Results of the samples are valid. Conditions of Rule 5 have been met.

- Rule 5: The values of the $C_{T\ \text{Total NPM1}}$ (Sample) for all samples are within the input interval [23.84; 29.2].

NPM1 mutation detection

Detection of an NPM1 mutation in the samples is shown in Table 11.

Table 11. NPM1 mutation detection

	Mean C_T values with PPM		Screening	
Sample	Total NPM1	Mut NPM1	Mutation Detected	Type
1	26.46	27.66	Yes	MutA
2	26.12	32.73	Yes	MutA
3	27.68	29.12	Yes	MutB
4	28.78	34.92	Yes	MutB
5	26.36	27.49	Yes	MutD
6	26.56	33.73	Yes	MutD
7	25.81	27.06	Yes	Not typed
8	26.89	27.87	Yes	Not typed
9	25.64	Undetected	No	NA

Samples positive for NPM1 mutation are detected: $C_{T\ Mut\ NPM1}$ (Sample).
Conditions of Rule 6 have been met for 8 of 9 samples.

- Rule 6: A sample is positive for an NPM1 mutation if a C_T value is obtained with PPM-Mut NPM1.

NPM1 mutation type

The procedure used to assign the NPM1 mutation type to samples positive for an NPM1 mutation is shown in Table 12.

Table 12. Typing of NPM1 mutation as MutA, MutB, or MutD

	Mean C_T values with PPM				
Sample	Mut NPM1	NPM1 MutA	NPM1 Mut B	NPM1 MutD	Type
1	27.66	28.29	Undetected	38.37	MutA
2	32.73	32.78	Undetected	Undetected	MutA
3	29.12	Undetected	29.79	Undetected	MutB
4	34.92	Undetected	33.57	Undetected	MutB
5	27.49	36.28	Undetected	27.96	MutD
6	33.73	Undetected	Undetected	33.54	MutD
7	27.06	37.23	38.7	Undetected	Not typed
8	27.87	38.91	Undetected	Undetected	Not typed
9	Undetected	Undetected	Undetected	Undetected	NA

If a sample has C_T values for more than one of the primers and probe mixes for detecting MutA, MutB, or MutD mutations, the lowest of these C_T values for the sample is used to assign the mutation type. See sample 1 and sample 5 in Table 12. Samples may be genotyped providing the conditions of Rule 7 are met. Conditions of Rule 7 have been met in 6 of 8 samples.

- Rule 7: The NPM1 mutation type of a sample is determined by the lowest C_T value obtained with PPM-NPM1 MutA, PPM-NPM1 MutB, and PPM-NPM1 MutD providing
  $C_{T\ \text{NPM1 MutA, MutB, or MutD}}$ (Sample) ≤ $C_{T\ \text{Mut NPM1}}$ (Sample) + 4.4.

For sample 7 and sample 8, the $C_{T\ \text{NPM1 MutA}}$ (Sample) values (37.23 and 38.91) are greater than the $C_{T\ \text{Mut NPM1}}$ (Sample) values + 4.4. These values are 31.46 and 32.27 respectively.

Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this

handbook or sample and assay technologies (for contact information, see "Contact Information", page 28).

	Comments and suggestions
NPM1 wild-type control not detected with PPM-Total NPM1	
a) Pipetting errors or omitted reagents; tube or well inversions	Check pipetting scheme and the setup of the reaction. Repeat the PCR run.
b) Inappropriate storage of kit components	Aliquot reagents for storage. Store the *ipsogen* NPM1 Muta*Screen* Kit at –15 to –30°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling", page 8. Avoid repeated freezing and thawing.
NPM1 mutated positive control not detected with PPM-Mut NPM1, PPM-NPM1 MutA, PPM-NPM1 MutB, or PPM-NPM1 MutD	
a) Pipetting errors or omitted reagents; tube or well inversions	Check pipetting scheme and the setup of the reaction. Repeat the PCR run.
b) Inappropriate storage of kit components	Aliquot reagents for storage. Store the *ipsogen* NPM1 Muta*Screen* Kit at –15 to –30°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling", page 8. Avoid repeated freezing and thawing.
No signal, even in controls	
a) In Rotor-Gene Q instruments, no reaction tube in position 1	Take care to always place a sample to be tested in position 1 of the rotor.
b) Pipetting errors or omitted reagents	Check pipetting scheme and the setup of the reaction. Repeat the PCR run.

	Comments and suggestions
c) Inappropriate storage of kit components	Aliquot reagents for storage. Store the *ipsogen* NPM1 Muta*Screen* Kit at –15 to –30°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling", page 8. Avoid repeated freezing and thawing.
d) Incorrect detection channel has been chosen	Set channel to Green channel or 530 nm/640 nm.
e) No data acquisition program	Check the cycle program. Select acquisition mode "Single" at the end of each annealing segment of the PCR program.
Fluorescence intensity varies	
a) Pipetting errors	Check pipetting scheme and the setup of the reaction. Vortex and spin all reagents after thawing. Repeat the PCR run.
b) Insufficient centrifugation of the tubes or plate	Always centrifuge tubes or plates loaded with the reaction mix as described in the specific operating manual of the apparatus.
Inconsistent C_T values between duplicates	
a) Pipetting errors or cross-contamination	Check pipetting scheme and the setup of the reaction. Repeat the PCR run.
b) Low mutation load	Always check the DNA quality (OD_{260}/OD_{280}) and concentration before starting.

	Comments and suggestions
Fluorescence intensity is too low	
a) Inappropriate storage of kit components	Aliquot reagents for storage. Store the *ipsogen* NPM1 Muta*Screen* Kit at –15 to –30°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling", page 8. Avoid repeated freezing and thawing.
b) Very low initial amount of target DNA	Check the amount of sample DNA. Always check the DNA quality (OD_{260}/OD_{280}) and concentration before starting. **Note**: Depending of the chosen method of DNA preparation, inhibitory effects may occur.
Signal absent or low in samples but okay in controls	
Inhibitory effects of sample material caused by insufficient purification	Always check the DNA quality (OD_{260}/OD_{280}) and concentration before starting. Repeat DNA preparation.
Negative (H_2O) control is positive	
Cross-contamination, reagent contamination, instrument error, well or capillary inversion, or probe degradation	Replace all critical reagents. Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carry over contamination. Keep primers and probe mixes protected from light. Check for false positives on fluorescence curves. Check the setup of the reaction.

Quality Control

This kit is manufactured according to ISO 13485 standard. Certificates of analysis are available on request at www.qiagen.com/support/.

References

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Symbols

The following symbols may appear on the packaging and labeling:

<N>	Contains reagents sufficient for <N> reactions
	Use by
	Catalog number
	Lot number
	Material number
	Temperature limitation
	Manufacturer
	Consult instructions for use

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Ordering Information

Product	Contents	Cat. no.
ipsogen NPM1 Muta*Screen* Kit (24)	For 24 reactions: Wild-type NPM1 Control, Mutated NPM1 Control , Primers and Probe Mix PPM-Total NPM1, Primers and Probe Mix PPM-Mutated NPM1, Primers and Probe Mix PPM-NPM1 MutA, Primers and Probe Mix PPM-NPM1 MutB, Primers and Probe Mix PPM-NPM1 MutD	677013
Rotor-Gene Q — for outstanding performance in real-time PCR		
Rotor-Gene Q 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001580
Rotor-Gene Q 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001650

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